Impact of Oncogenic Targets Controlled by Tumor-Suppressive miR-30a-5p in Pancreatic Ductal Adenocarcinoma

最終試験の結果の要旨

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主査および副査の５名は、令和３年12月23日、学位申請者 プラモド・ネパール 君に面接し、学位申請論文の内容について説明を求めると共に、関連事項について試問を行った。具体的には、以下のような質疑応答がなされ、いずれについても満足すべき回答を得ることができた。

(Q1) Why is the number pancreatic cancer patients increasing worldwide?
Ans: There is a continuous increase in the risk factors of pancreatic ductal adenocarcinoma (PDAC) such as obesity, chronic pancreatitis, diabetes, multiple viral and bacterial infections, and increased consumption of alcohol, cigarettes. This might be the reason for increasing incidence of pancreatic cancer worldwide.
(Q2) Is there any correlation between *KRAS* mutation *miR-30a-5p?*
Ans: We did not investigate the relation between *miR-30a* and *KRAS* mutation. However, it has been reported that *miR-30a-5p* inhibits tumorigenesis of *KRAS* mutant colorectal cancer cells by directly inhibiting *KRAS* and malic enzyme 1. *miR-30a-5p* may be an important therapeutic candidate for pancreatic cancer because of a high rate of *KRAS* mutations.
(Q3) Is there any correlation between *KRAS* mutation and *RRM2*?
Ans: It has been reported that *RRM2* is upregulated via *KRAS* in colorectal cancer cells. Therefore, it is possible that *KRAS* mutations are also involved in increased expression of RRM2 in pancreatic cancer.
(Q4) What is more important for PDAC patient prognosis, the biological behavior of *RRM2* or *RRM2* induced chemoresistance?
Ans: I think the biological behavior of *RRM2* with oncogenic activity as well as its role in chemoresistance is important in prognosis in PDAC patients. However, association of high expression of *RRM2* and chemoresistance is described more directly whereas *RRM2* mediated oncogenic behavior is still being investigated.
(Q5) Since *RRM2* influences the effectiveness of chemotherapy, can it be a predictor of chemoresistance and help to choose chemotherapy regimen?
Ans: Yes, *RRM2* can possibly be a predictor of response to chemotherapy and may help choose treatment regimen. In different studies, the level of *RRM2* expression is correlated with gemcitabine resistance and the sensitivity to gemcitabine treatment can be predicted by measuring *RRM2* expression in patients with PDAC and non-small cell lung cancer. However, more extensive research in PDAC patients is necessary to validate the effect of *RRM2* overexpression and incidence of chemoresistance.
(Q6) Why did you choose *miR-30a-5p*?
Ans: In PDAC miRNA expression profile *miR-30a-5p* and *miR-30a-3p* are significantly downregulated. In our previous project, we revealed that both miRNAs act as tumor-suppressor miRNAs in PDAC. Our previous study explored the role of *miR-30a-3p* in PDAC. So, this time we chose *miR-30a-5p* to explore its role in PDAC pathogenesis.
(Q7) The figure in your slide showed that *miR-30a-5p* is strongly downregulated in PDAC cell lines. I don't think the cell line is sufficiently stable for *miR-30a-5p*, but how about it?
Ans: The intracellular stability of *miR-30a-5p* in PDAC cells has not been investigated in this study. However, the *miR-30c-2-3p*, which is also strongly downregulated in pancreatic cancer, binds to Ago2 and is taken up in the same PDAC cells. It was confirmed that it would be retained for 3 days. The transfected miRNA is considered to be functioning in pancreatic cancer cells. It has been reported that downregulation of microRNA involves the degradation of microRNA by methylation and the adsorption of microRNA by long non-cording RNA.
(Q8) RRM2 protein can affect the cell migration, invasion and proliferation. How about the cell viability itself which you conducted transfection of miR-30a-5p?
Ans: I have not investigated the cell viability itself, but the number of PDAC cells after 72 hours of transfection were enough to perform the functional assays as per protocol, thus I think the PDAC cells viable enough to conduct functional assays.
(Q9) In Figure 8, could you explain the figure?
Ans: The figure represents the enrichment of gene sets. The primary result of the gene set enrichment analysis is the enrichment score (ES), which reflects the degree to which a gene set is overrepresented at the top or bottom of a ranked list of genes. The middle portion of the plot (like bar code) shows where the members of the gene set appear in the ranked list of genes.

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(Q10) In Figure 4 multivariate analysis, do you think age and sex should be analyzed as an important factor for PDAC prognosis?
Ans: In this study, we analyzed the prognosis only taking account of the Gene expression, Pathological stage, LN stage and Tumor stage which showed significant differences in univariate analysis. These are already established determinants of survival. It is a good idea to find out whether age and sex also influenced the prognosis in PDAC patients.
(Q11) Why didn't you focus on other two genes rather than *RRM2*?15
Ans: The expression of *RRM2* following the transfection of *miR-30a-5p* was most downregulated compared to *CBFB* and *AHNAK* in our own experiment. We believed *miR-30a-5p* has more suppressing effect on *RRM2* than other two genes. Thus, we selected *RRM2*.
(Q12) The expression of RRM2 protein in IHC looks like heterogeneous. What is your opinion?
Ans: Yes, the expression of *RRM2* in pancreatic cancer cells on IHC varied at the protein level. I presume that the variability in protein levels is due to tumor heterogeneity.
(Q13) In Figure 5, when compared to the height of bar representing mock, the height of bar representing control looks different. What is your opinion?
Ans: The height of bar of the mock and control looks different in Figure 5. However, there was no statistical difference between them. In the future, we will repeat experiments in such cases to obtain more precise results.
(Q14) In Figure 6, the proliferation is not suppressed compared with invasion and migration assay. Why? In GSEA, did you find any genes related to cell migration?
Ans: During invasion and migration assays we utilized the surviving cells and discarded the dead cells. This might result in increased suppression of migration and invasion. We did not find any genes which increase cell migration enriched in GSEA analysis. However, previous studies have reported that overexpression of *RRM2* promoted tumor migration in nasopharyngeal carcinoma, glioblastoma, and breast cancer. This may be the reason for suppression of cell migration in knockdown assays in pancreatic cancer cells too.
(Q15) Is *miR-30a-5p* a guide strand or passenger strand?
Ans: We have not conducted direct experiments to investigate which one of *miR-30a-5p* and-*3p* is more likely to be incorporated into the cells of pancreatic cancer cell lines and which is the guide strand. Based on the results of deep sequencing shown in miRBase, I thought that *miR-30a-5p* also functions as a guide strand in pancreatic cancer.
(Q16) Are there any genes which are common targets of *miR-30a-5p* and *miR-30a-3p* among the genes shown in Table 1?
Ans: We found no common target genes of both *miR-30a-5p* and *miR-30a-3p*.
(Q17) Did you investigate whether *AHNAK*, *CBFB* and *DCBLD1* also bind to *miR-30a-5p*?
Ans: This time we did not investigate whether other 3 genes have binding site in *miR-30a-5p*.
(Q18) In Figure 8, high expression of *RRM2* is related to enriched G2/M checkpoint related genes. Do you think *RRM2* knockdown decreases cell proliferation owing to G2/M checkpoint genes? How do you explain?
Ans: High expression of *RRM2* is reported to be associated with imbalance in dNTP pool resulting in increased base incorporation during DNA synthesis, decreased proofreading and increased in genomic instability leading to cell cycle arrest required for DNA repair. This may be one of the possible reasons for enrichment of G2/M related genes during increased *RRM2* expression. The knockdown of *RRM2* in glioma cells is reported to decrease the cell proliferation, increase the apoptosis of the cells and increase the percentage of the cells in G2 phase. This might be the result of cell cycle arrest owing to lack of dNTP pool during S phase. The enzymatic activity and gene regulatory activity of *RRM2* is complex, and the effect of *RRM2* in cell proliferation in PDAC requires further investigation.
(Q19) Did you perform gene expression analysis in cells after *siRRM2* transfection?
Ans: We did not perform GSEA analysis after transfecting *siRRM2*. It is a good idea to check whether the G2/M checkpoint genes are downregulated following *siRRM2* transfection.
(Q20) In Figure 1, Why did you use GSE15471 dataset?
Ans: GSE15471 data set contains matched pairs of 36 cancer samples and 36 normal samples. It has been being used in our lab previously. Therefore, we selected this dataset.
(Q21) What are the functions of *RRM2* other than the enzymatic activity?
Ans: Other than enzymatic activity, *RRM2* is involved in genetic regulation also. It utilizes various regulatory pathways like *Wnt*-signaling pathways, *Nf-kB* pathways and AKT signaling pathways. It is also involved in regulation of various genes like *VEGF*, *EGFR*, *MMP-9*, E-cadherin, and also contributes to epithelial-mesenchymal transformation.
(Q22) How did you divide *RRM2* express into high expression group and low expression group to perform GSEA analysis?
Ans: We extracted data from the TCGA database and performed GSEA analysis by dividing the *RRM2* expression of the data into a high expression group and a low expression group at the median.

以上の結果から、５名の審査委員は申請者が大学院博士課程修了者としての学力・識見を有しているものと認め、博士（医学）の学位を与えるに足る資格を有するものと認定した。